Effect of starvation on survival of three bacterial isolates from an arctic soil

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Three isolates, a *Pseudomonas* sp., a *Bacillus* sp. and an *Arthrobacter* sp., which had been isolated from a meadow soil at Devon Island, Canada, were subjected to starvation under varying conditions. The viabilities of the three isolates during starvation for 30 days in a carbon-free medium was assessed after the organisms had been grown continuously at varying rates in carbon- and nitrogen-limited media at 5 and 15°C. *Pseudomonas* M216 was the most resistant to starvation stress, *Bacillus* M153 the least, and *Arthrobacter* M51 was intermediate in its response. Cells grown and starved at 5°C survived longer than those at 15°C. Carbon-limited *Bacillus* and *Arthrobacter* cells grown at high rates prior to starvation survived longer than those grown slowly, while in nitrogen-limited *Arthrobacter* the reverse was observed. The pattern of endogenous metabolism of the three isolates during starvation at 15°C for 10 days was similar to that observed in other organisms. Levels of endogenous substrates such as carbohydrate and protein showed a rapid decrease in the initial 20 h of starvation, followed by a gradual decline over the remainder of the starvation period. The rates of endogenous metabolism of the isolates were positively correlated with their survival rates during starvation.

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Nous avons soumis au jeûne sous diverses conditions, trois isolats qui ont été isolés d'un sol de prairie dans l'île de Devon au Canada; ces isolats appartiennent aux genres *Pseudomonas*, *Bacillus* et *Arthrobacter*. Après avoir fait pousser les organismes en culture continue à divers taux de croissance dans un milieu limité en carbone et en azote à 6 et à 15°C, nous avons déterminé les viabilités des trois isolats au cours d'une période de jeûne de 30 jours dans un milieu sans carbone. *Pseudomonas* M216 est le plus résistant et *Bacillus* M153 le moins résistant au stress du jeûne, quant à *Arthrobacter* M51, il donne une réponse intermédiaire. Les cellules qui ont poussé et jeûné à 5°C survivent plus longtemps que celles à 15°C. Les cellules de *Bacillus* et d'*Arthrobacter* qui ont pourré à un taux de croissance élevé sur un milieu limité en carbone avant de jeûner survivent plus longtemps que cells qui ont poussé lentement; nous avons observé le phénomène inverse avec des cellules d'*Arthrobacter* limitées en azote. Le modèle du métabolisme endogène de ces trois isolats, au cours d'un jeûne de 10 jours à 15°C, est semblable à celui observé chez d'autres organismes. Les niveaux de substrats endogènes tel que hydrates de carbone et protèines décroissent rapidement dans les 20 premières h du jeûne, ceci est suivi par un déclin graduel pour le reste de la période de jeûne. Nous avons établi und corrélation positive entre le taux du métabolisme endogène des isolats et leur survie au cours du jeûne.

[Traduit par le journal]

Introduction

Starvation is one of the principal stresses which microorganisms may undergo in their natural environment. In two soil systems Gray and Williams (1971) and Shields *et al.* (1973) determined that there was sufficient incoming energy to permit only several divisions per year of the microbial populations, assuming that a portion of the available energy was required for cell maintenance (Marr *et al.* 1963). Many microorganisms form spores or dormant bodies to aid in their survival during unfavourable conditions but for most bacteria the vegetative cell is the sole means of survival (Gray 1976). When exogenous energy sources are low or absent, the cell's requirement for energy to maintain its basic functions must be supplied endogenously by utilization of specialized carbon and energy reserves (Dawes and Senior 1973) or by utilization of basic cell components. Viability during starvation conditions has been correlated wtih the adenylate energy charge (Chapman *et al.* 1971; Montague and Dawes 1974). The ability of an organism to survive during starvation under laboratory conditions has been related to the levels of endogenous substrate present, to the rate at which these substrates were utilized, and ultimately, to the ability to maintain adequate levels of ATP for necessary cell functions (Dawes 1976). Environmental factors prior to and during starvation have also been shown to affect survival rate (Postgate 1976; Postgate and Hunter 1962).

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While temperature is probably the primary limiting factor in tundra ecosystems, bacteria in tundra soils may also be subject to starvation as these soils are generally poor and low in available nutrients (Bliss 1975; Bunnell *et al.* 1975). The ability of these organisms to persist under low nutrient conditions may be an important factor, therefore, in determining their success in this environment. As part of a study of the growth and survival characteristics of three bacterial isolates from a tundra soil at Devon Island, Canada, we investigated the abilities of these isolates to survive starvation. The effects of temperature, growth rate, and nutrient limitation prior to starvation on survival during 30 days and the utilization rate of several endogenous substrates during the initial 10 days of starvation were studied, and the results are presented here.

Materials and Methods

Bacterial Strains

Three strains chosen for study, *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51, were isolated from the hummocky sedge – moss meadow at Truelove Lowland, Devon Island, N.W.T., Canada, and characterized as previously described (Nelson and Parkinson 1978).

Growth Conditions prior to Starvation

The three isolates were grown in duplicate in continuous culture under carbon or nitrogen limitation at 5 and 15°C as described in Nelson (submitted Can. J. Microbiol.). Specific growth rates of about 0.04 h^{-1}, 0.02 h^{-1}, and 0.01 h^{-1} were studied at each treatment. Cultures were allowed to grow until at least three culture volumes had passed through the culture vessel to ensure that a steady state had been achieved.

Preparation of the Starved Suspensions

Cell suspensions for the determination of viability were prepared in duplicate from each treatment. Twenty-millilitre aliquots were harvested aseptically from each continuous culture vessel, centrifuged at 10 000 rpm for 10 min at 4°C, and washed twice with a carbon-free medium modified from Chen and Alexander (1972) of the following composition: 2 g KNO_3, 0.8 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl in 1000 ml of distilled water. The pellets were then resuspended into 250-ml Erlenmeyer flasks containing 100 ml of the carbon-free medium. Prior to use the flasks were soaked 24 h in Count-off (New England Nuclear) to remove any residual organic material. Samples taken from the 15°C continuous-culture treatments were then incubated at 15°C in a New Brunswick Psychrotherm and shaken at 100 rpm. Samples from the 5°C continuous-culture treatments were incubated unshaken at 5°C in a controlled temperature incubator.

Suspensions for the determination of intracellular constituents and oxygen-uptake rates were prepared from cells grown at D = 0.02 h^{-1} at 15°C under carbon- and nitrogen-limiting conditions. The carbon-limited medium in this case was medium K (Nelson and Parkinson 1978) in which the glucose concentration was reduced to 0.1% and yeast extract (0.02%) supplemented the medium. Aliquots of 250 ml were harvested from each growth vessel, centrifuged at 10 000 rpm for 15 min, and the two replicates of each isolate were pooled, washed twice with carbon-free medium, and resuspended in 100 ml of the carbon-free medium. Twenty-millilitre portions were transferred aseptically to precleaned 250-ml Erlenmeyer flasks containing 80 ml of the sterile carbon-free medium and the flasks incubated at 15°C in the New Brunswick Psychotherm and shaken at 100 rpm.

Analytical Procedures

Viability of the starved suspensions was determined on 0, 1, 2, 5, 10, 20, and 30 days after the initiation of starvation conditions by the slide-culture method as described in Nelson (submitted Can. J. Microbiol.).

Analyses of intracellular components and oxygen-uptake rates were performed in duplicate on days 0, 1, 2, 3, 4, 5, and 10 after the initiation of starvation. Dry weight, protein, hexose carbohydrate, ribonucleic acid, and ATP were determined as described in Nelson (submitted Can. J. Microbiol.). Oxygen-uptake rates of 3-ml aliquots of the starved cell suspensions were measured in a Gilson differential respirometer at 15°C with shaking at 70 oscillations per minute. Viability of these suspensions was also determined by the slide-culture method during the 10-day starvation period.

Results

Effect of Growth Temperature, Growth Rate, and Nutrient Limitation on Survival of Devon Island Bacteria during Starvation

Viability was measured over a 30-day starvation period for each isolate previously grown in carbon- or nitrogen-limited continuous culture at 15 or 5°C at three dilution rates. Representative survival curves obtained from several of these experiments (carbon- or nitrogen-limited cultures grown at 15°C at dilution rates of 0.04 h^{-1} and 0.01 h^{-1}) are presented in Fig. 1. In all cases *Pseudomonas* M216 was most resistant to starvation, *Bacillus* M153 least resistant, and *Arthrobacter* M51 intermediate in response. Spore formation was not observed in *Bacillus* M153 under these conditions. Data obtained at 5°C were similar to those presented in Fig. 1 but the plots had a shallower slope, that is; survival during starvation was prolonged slightly in populations previously grown and then starved at a lower temperature. This effect was particularly marked in *Bacillus* M153, the most susceptible to starvation stress.

Only data obtained for cells previously grown at the highest (0.04) and lowest (0.01) dilution rates are presented in Fig. 1. Plots of cells grown at intermediate growth rates generally fell between or in the same range as those presented. Although no consistent pattern of survival with growth rate was evident among the three organisms several observations were made. In general, the longevity of *Pseudomonas* M216 and the rapid death rate of *Bacillus* M153 made the effects of growth rate insignificant. In *Arthrobacter* M51 previously carbon-limited cells grown at high dilution rates survived longer than those which were grown slowly (also evident in *Bacillus* M153) while previously nitrogen-limited cells of *Arthrobacter* exhibited the opposite response: cells grown slowly

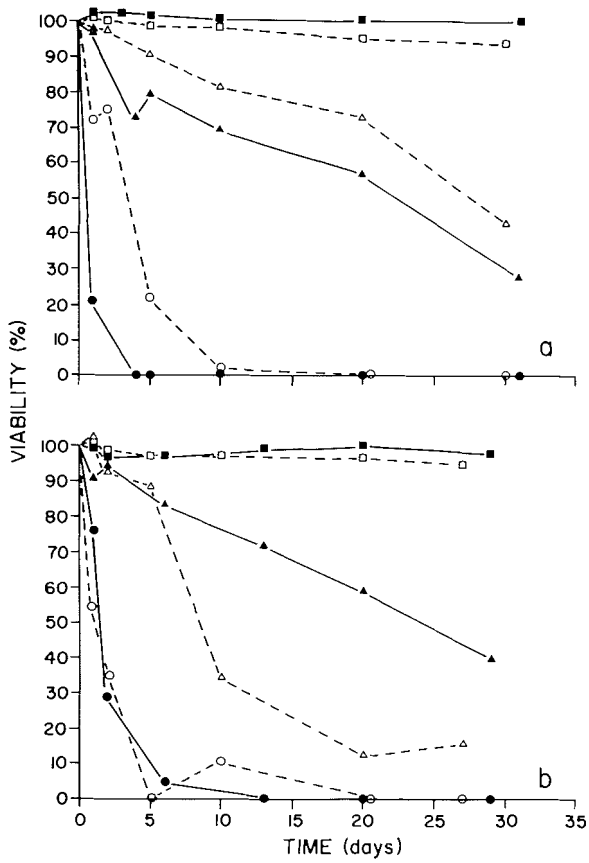

FIG. 1. Viabilities of *Pseudomonas* M216 (□, ■), *Bacillus* M153 (○, ●) and *Arthrobacter* M51 (△, ▲), during starvation in carbon-free medium following growth in continuous culture in (*a*) carbon-limited medium at 15°C and (*b*) nitrogen-limited medium at 15°C. Dilution rates prior to starvation were 0.04 h^{-1} (broken line and open symbols) and 0.01 h^{-1} (solid line and closed symbols). Values presented are the mean of two replicates.

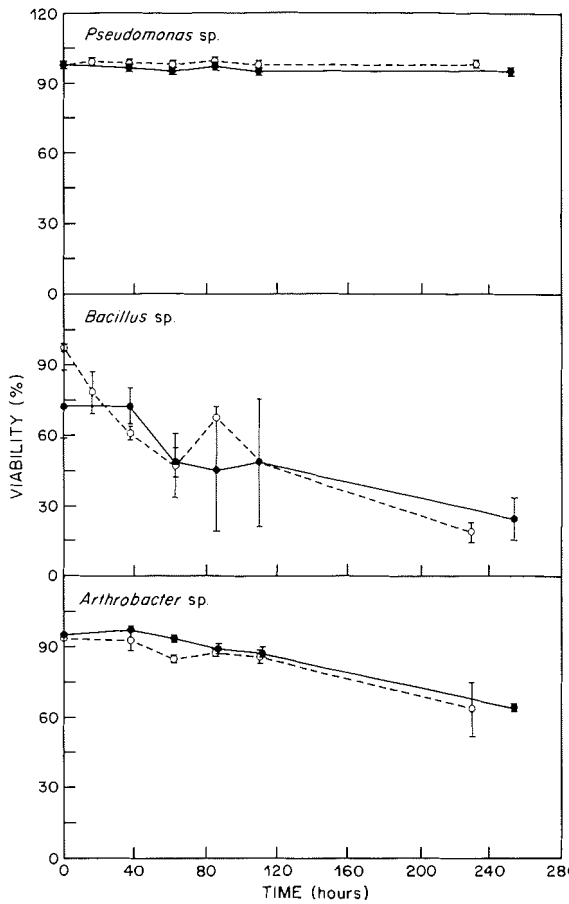

FIG. 2. Viabilities (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Prior to starvation cells were grown in continuous culture at D = 0.02 h^{-1} in carbon-limited medium (solid line) or nitrogen-limited medium (broken line).

survived longer than those grown at high rates. In general, there did not appear to be a significant difference in survival time between cells which had been carbon- or nitrogen-limited prior to starvation.

Effect of Starvation on Levels of Endogenous Substrates and Rates of Endogenous Respiration

Studies of the loss of intracellular constituents, of oxygen-uptake rates, and viability during starvation at 15°C for 10 days were carried out on populations of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 which had been grown in carbon- and nitrogen-limited continuous cultures at a dilu-

studies reported above. There was no significant difference between carbon- or nitrogen-limited cells, *Pseudomonas* M216 remained close to 100% viable, *Bacillus* M153 showed a rapid decline in viability, and *Arthrobacter* M51 a slower loss of viability. Dry weights (Fig. 3) decreased sharply in the first 20 to 40 h and more slowly if at all thereafter. Cells which had been carbon-limited exhibited a greater loss in dry weight than nitrogen-limited cells and *Bacillus* M153 showed the greatest dry weight loss during the study. Q_{O_2} values (Fig. 4) of carbon-limited cells were initially high and declined rapidly in the first 20 h, coincident with the rapid dry weight loss. Nitrogen-limited cells exhibited a low oxygen-uptake rate initially and Q_{O_2}

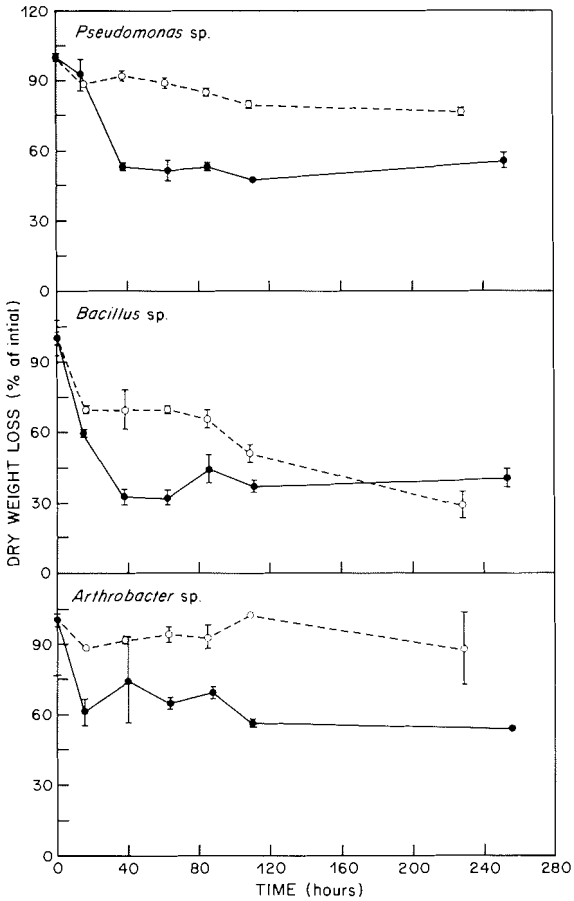

FIG. 3. Dry weight losses (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Prior to starvation cells were grown in continuous culture at $D = 0.02\,h^{-1}$ in carbon-limited medium (solid line) or nitrogen-limited medium (broken line).

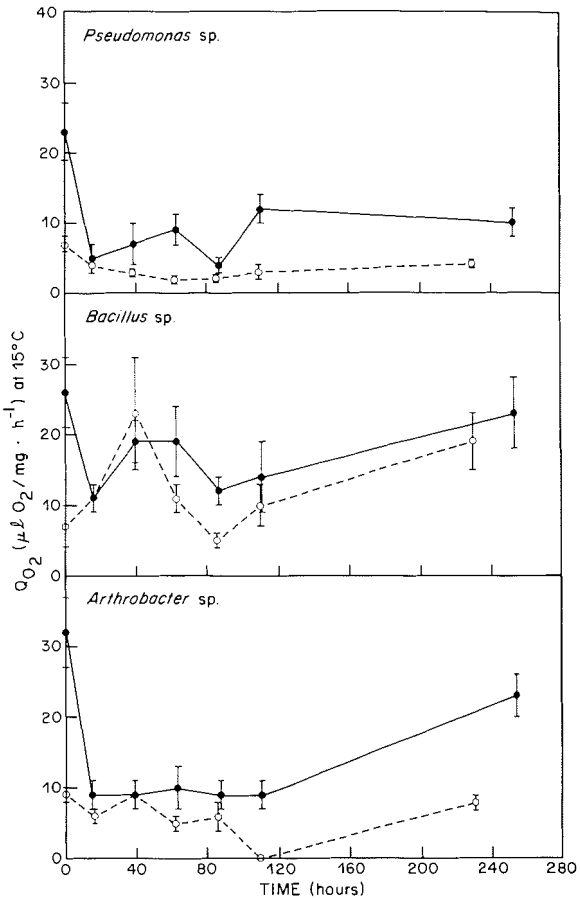

FIG. 4. Oxygen-uptake rates (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Cells were grown in continuous culture at $D = 0.02\,h^{-1}$ in carbon-limited (solid line) or nitrogen-limited medium (broken line) prior to starvation.

crease in Q_{O_2} of *Bacillus* and *Arthrobacter* on the final day of the study may possibly be attributed to the organisms' metabolism of substrates released from dead cells.

Measurements of protein, hexose carbohydrate, RNA, and ATP during the starvation period showed a similar pattern of decline. Protein (Fig. 5) declined slowly in both carbon- and nitrogen-limited cells except in carbon-limited *Pseudomonas* M216 which exhibited a sharp decrease in protein in the first 20 h of starvation. Nitrogen-limited cells showed higher initial concentrations of hexose carbohydrate than carbon-limited cells (Fig. 6) but these decreased rapidly in the first 20 h. RNA levels declined gradually during starvation (Fig. 7) with some variation initially in *Bacillus* M153 and *Arthrobacter* M51. There was a gradual decline in ATP content during starvation of *Pseudomonas* and *Arthrobacter* but in *Bacillus* which had a high initial content of ATP there was a sharp decrease in the first 40 h to a barely detectable level (Fig. 8).

Discussion

Survival of the three isolates from Devon Island during starvation, after growth under varying conditions in continuous culture, was similar to that found in batch culture studies (Nelson 1977). The projected 50% survival times of the three isolates at 30°C are compared in Table 1 with other organisms (modified and expanded from Boylen and Ensign (1970*a*)). Decreasing the temperature of starvation by a factor of two has been shown to prolong survival by a factor of 6 to 8 (Postgate and Hunter 1962; Thomas and Batt 1968) and to permit comparison with other studies this latter factor was used to

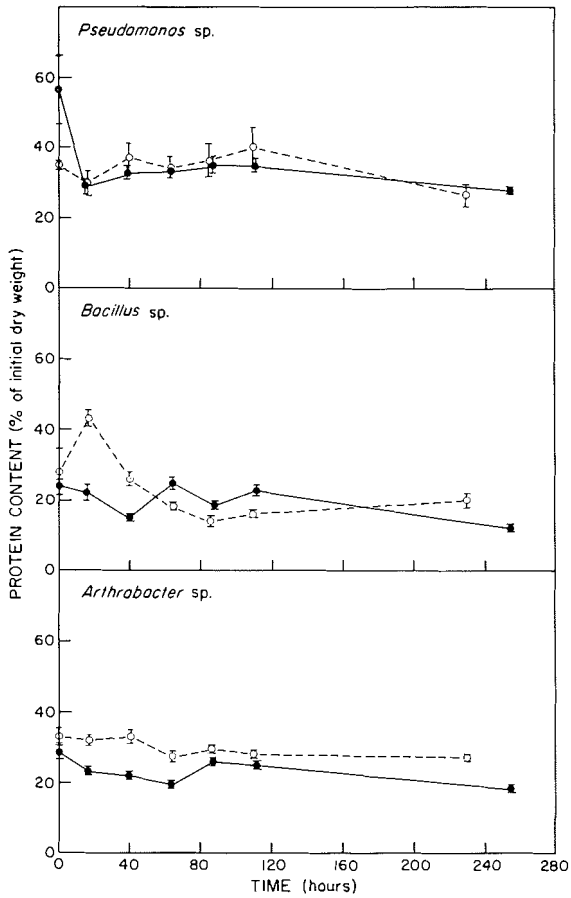

FIG. 5. Protein levels (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Cells were grown in continuous culture at $D \pm 0.02\,h^{-1}$ in carbon-limited (solid line) or nitrogen-limited medium (broken line) prior to starvation.

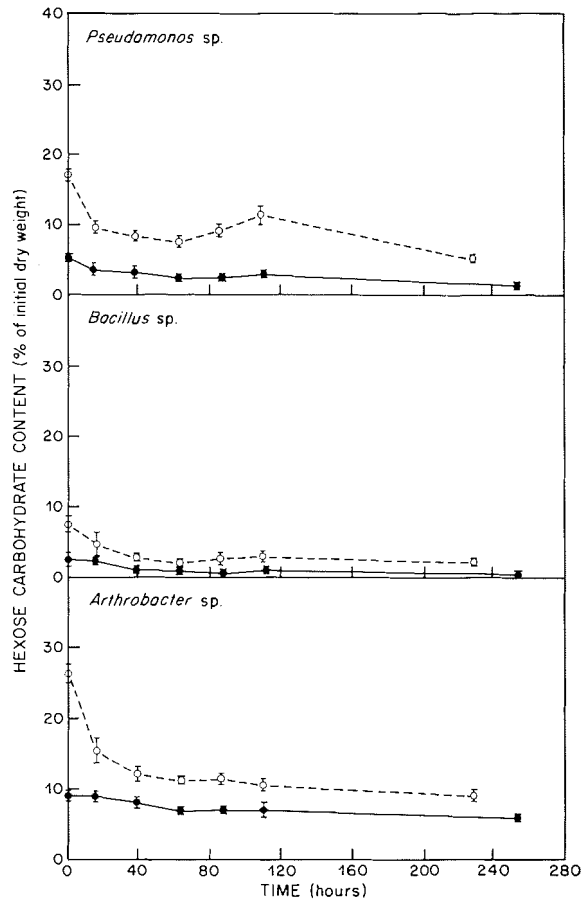

FIG. 6. Hexose carbohydrate levels (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Cells were grown in continuous culture at $D = 0.02\,h^{-1}$ in carbon-limited (solid line) or nitrogen-limited (broken line) medium prior to starvation.

estimate the survival times of the Devon Island isolates from the data obtained at 15°C. If this factor is applicable to our isolates then *Pseudomonas* M216 is among the most resistant of organisms studied, *Bacillus* M153 one of the most susceptible to death due to starvation, and *Arthrobacter* M51 intermediate in its ability to survive starvation. It is interesting that *Bacillus* M153 did not exhibit any appreciable spore formation under the starvation conditions studied.

Several workers (Postgate and Hunter 1962; Thomas and Batt 1968) have demonstrated that cultural conditions prior to starvation and the environmental conditions during starvation can profoundly affect survival of microorganisms. Only three factors were examined in the present study: temperature, growth rate prior to starvation, and nutrient limitation prior to starvation. Cells starved at 5°C survived longer than those at 15°C. Postgate and Hunter (1962) reported an increase in survival time of *Enterobacter aerogenes* as temperature was decreased to 20°C but at 10°C survival was less than 20°C. The mesophilic nature of the organism or injury due to cold shock may have accounted for these results. In the present study organisms starved at 5°C had also been grown at 5°C so that the population's metabolism was already acclimatized to a lower temperature. Novitsky and Morita (1977) were able to demonstrate viable cells in populations of a psychrophilic marine vibrio which had been starved at 5°C for more than 1 year. It seems likely that, for organisms adapted for growth at low temperature, resistance to starvation will increase with decreasing temperature within the limits imposed by freezing.

The effects of prior growth rate and nutrient limitation on starvation survival were more complex. The enhanced survival of previously fast-

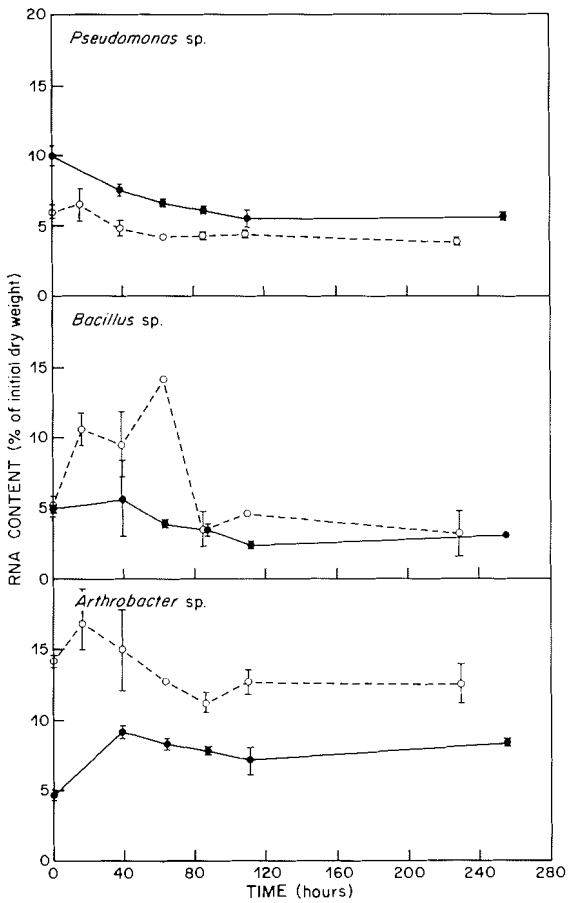

FIG. 7. RNA levels (± standard error) of *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Cells were grown in continuous culture at $D = 0.02\ h^{-1}$ in carbon-limited (solid line) or nitrogen-limited (broken line) medium prior to starvation.

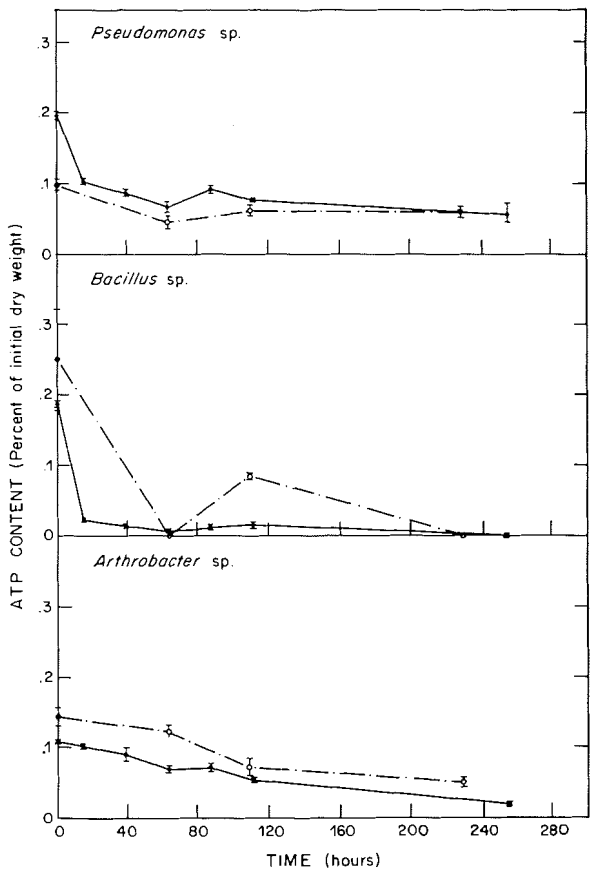

FIG. 8. Levels of ATP in *Pseudomonas* M216, *Bacillus* M153, and *Arthrobacter* M51 during starvation at 15°C. Cells were grown in continuous culture at $D = 0.02\ h^{-1}$ in carbon-limited (solid line) or nitrogen-limited (broken line) medium prior to starvation.

growing cells as opposed to slow-growing cells, particularly for carbon-limited populations has been reported before (Postgate and Hunter 1962). The enhanced survival of slowly grown, nitrogen-limited *Arthrobacter* M51 is similar to that reported for other organisms (Dawes and Senior 1973; Harrison 1961) and may be related to the higher levels of carbohydrate reserves accumulated by this organism under these conditions (Nelson, submitted to Can. J. Microbiol.). The significance of such reserves for starvation will be discussed further below.

A general pattern of endogenous metabolism, dependent on the concentrations of endogenous substrates which in turn is determined by the nutritional status of the cell prior to starvation, has been observed in bacteria (Boylen and Ensign 1970*b*; Dawes and Ribbons 1964; Robertson and Batt 1973). This pattern was one in which specialized reserve materials, if present, were rapidly degraded initially with concomitant high Q_{O2} values (Dawes and Ribbons 1964), followed by degradation of protein and RNA (Dawes and Ribbons 1964; Robertson and Batt 1973). The specialized reserves tended to have a sparing action on RNA and protein degradation although this was not the case in *Sarcina lutea* (Burleigh and Dawes 1967). DNA and fatty acids (other than poly-β-hydroxybutyrate (PHB)) were not degraded extensively during starvation. An exception was noted recently for a marine vibrio (Novitsky and Morita 1977) which exhibited significant DNA degradation during starvation. The importance of the specialized reserves such as glycogen or PHB for prolongation of survival during starvation seems questionable, particularly for organisms which exhibit a rapid degradation of these endogenous substrates with the onset of starvation. A pattern of slow degradation of endogenous substrates and low oxygen-consumption

TABLE 1. Comparison of 50% survival times[a] of starved bacteria

Organism	50% survival time, h	Reference
Sphaerotilus discophorus	12	Stokes and Parson 1968
Starvation-susceptible soil isolates	12	Chen and Alexander 1972
Bacillus M153[b]	16	This study
Streptococcus mitis	22	van Houte and Jansen 1970
S. lactis	30	Thomas and Batt 1968
Escherichia coli	36	Dawes and Ribbons 1965
Aerobacter aerogenes	45	Strange *et al.* 1963
Azotobacter agilis	50	Sobeck *et al.* 1966
Sarcina lutea	65	Burleigh and Dawes 1967
Pseudomonas aeruginosa	96	Mackelvie *et al.* 1968
Starvation-resistant soil isolate R5	100	Chen and Alexander 1972
Arthrobacter M51[b]	160	This study
Nocardia corallina	480	Robertson and Batt 1973
Pseudomonas M216[b]	1030	This study
Arthrobacter globiformis	1370	Luscombe and Gray 1974
Arthrobacter sp.	1680	Zevenhuizen 1966
Arthrobacter crystallopoietes	2400	Boylen and Ensign 1970*a*

[a]Time required for half of the population to die; the longest 50% survival time at 30–37°C reported in each study is presented.
[b]Fifty percent survival times at 30°C are estimated from 50% survival times obtained at 15°C by mean sof a correction factor.

rates appears to be correlated with increased viability during starvation and has most often been exhibited in soil isolates (Boylen and Ensign 1970*b*; Luscombe and Gray 1974; Robertson and Batt 1973) and a marine isolate (Novitsky and Morita 1977).

The utilization of endogenous substrates during starvation by the three Devon Island isolates resembled the general pattern described above. A rapid utilization of carbohydrate reserves in nitrogen-limited cells, coinciding with a rapid initial loss in dry weight was accompanied by a slow decrease in protein and RNA. Carbon-limited cells, lacking the initial carbohydrate reserves, degraded protein and RNA more rapidly initially and exhibited a higher dry weight loss which was correlated with the higher Q_{O_2} values measured. These apparent differences in rates of endogenous metabolism did not correlate with differences in viabilities between the two populations; carbon and nitrogen-limited cells exhibited similar survival patterns during the 10-day study period. However, differences in survival rates among the three organisms did correlate with their rates of endogenous metabolism. *Bacillus* M153 which was most susceptible to starvation, exhibited the highest Q_{O_2} values, the most rapid decrease in dry weight and the most rapid decrease in ATP levels. Q_{O_2} values of *Pseudomonas* M216, the most resistant of the three isolates studied, were higher than those obtained for other resistant organisms (Boylen and Ensign 1970*b*; Luscombe and Gray 1974; Robertson and Batt 1973) but the pattern of slow utilization of endogenous substrates was apparent.

While it is not possible to state precisely what factors cause the death of vegetative bacteria (Dawes 1976) it appears that the ability to establish a low rate of endogenous metabolism is associated with the ability to survive during starvation. In this regard organisms living in nutrient-poor environments such as soil or water have likely been selected, in part, for their starvation resistance. *Bacillus* M153, which was not commonly isolated at the Devon Island site (Nelson and Parkinson 1978), was also the least resistant to starvation stress. This organism may possibly survive in low numbers as a result of spore formation, which did not occur frequently under the particular conditions used in this study. *Pseudomonas* M216 and *Arthrobacter* M51, however, were better suited for survival in a nutrient-poor environment, a factor which may reflect the more frequent isolation of those genera in the meadow soil at Devon Island. The low temperatures characteristic of tundra soils will enhance starvation survival of these organisms in their natural environment.

Acknowledgements

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